Startle Reactivity and Anxiety Disorders: Aversive Conditioning, Context, and Neurobiology

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The aim of this article is to review studies on human anxiety using the startle reflex methodology and to apply the literature on context conditioning in rats to interpret the results. A distinction is made between cued fear (as in specific phobia), a phasic response to an explicit threat cue, and anxiety, a more sustained and future-oriented response not linked to a specific discrete cue. Experimentally, contextual fear, as opposed to cued fear, may best reflect the feeling of aversive expectation about potential future dangers that characterizes anxiety. Following a brief description of the neurobiology of cued fear and context conditioning, evidence is presented showing that anxious patients are overly sensitive to threatening contexts. It is then argued that the degree to which contextual fear is prompted by threat depends on whether the danger is predictable or unpredictable. Consistent with animal data, unpredictable shocks in humans result in greater context conditioning compared to predictable shocks. Because conditioning promotes predictability, it is proposed to use conditioning procedures to study the development of appropriate and inappropriate aversive expectations. Cued fear learning is seen as an adaptive process by which undifferentiated fear becomes cue-specific. Deficits in cued fear learning lead to the development of nonadaptive aversive expectancies and an attentional bias toward generalized threat. Lacking a cue for threat, the organism cannot identify periods of danger and safety and remains in a chronic state of anxiety. Factors that may affect conditioning are discussed. Biol Psychiatry 2002; 52:958–975 © *2002 Society of Biological Psychiatry*

Key Words: Fear conditioning, associative learning, context conditioning, startle, psychophysiology, anxiety

Introduction

Anxiety disorders present both overlapping and distinctive characteristics. One accepted distinction is that which exists between fear and anxiety. Fear is associated with a clearly identified *imminent* threat, whereas anxiety is a generalized fear without object (Marks 1987), an apprehensive anticipation of future *potential* threats (Barlow 2000). Recent animal studies have identified separate neural systems that may be associated with these two aversive states, the amygdala and the bed nucleus of the stria terminalis (BNST) (Davis 1998). The amygdala mediates fear responses to explicit threatening stimuli. The bed nucleus of the stria terminalis is an area adjacent to the amygdala that may play a role in chronic stress associated with more long-lasting aversive states not clearly linked to an explicit cue (Davis 1998). The objective of this review is to examine the relevance of these findings to human anxiety and anxiety disorders.

There are several animal models to explore aversive responses. The challenge has been to understand how the repertoires of these responses resemble the characteristics of human fear and anxiety. For example, ethoexperimental animal models based on the natural defensive repertoires of wild and laboratory rodents to predators present similarities with behavioral symptoms of anxiety disorders (Blanchard et al 1993). An important differentiation has been made between the patterns of defensive behaviors elicited by actual dangers and by potential or ambiguous threats as proxies for fear and anxiety, respectively (Blanchard et al 1993); however, to better understand psychological and neural mechanisms of fear and anxiety in humans, there is a need for investigative tools that can translate animal research into human experimentations. Combining Pavlovian aversive conditioning with the startle reflex methodology may be one of the most powerful ways to develop such cross-species studies.

Pavlovian aversive conditioning is the process by which initially neutral stimuli come to elicit defensive responses following their repeated association with an aversive event. Aversive conditioning procedures present several advantages for the study of human anxiety and anxiety disorders. Because brain structures activated during aversive conditioning procedures are highly preserved across species, inferences about neural structures involved in human fear and anxiety can be made based on animal studies (LeDoux 1995). In addition, conditioning studies in humans lag far behind animal research. Hence, the animal literature provides a rich source of theoretical information and empirical data on which to build and

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develop human research. Furthermore, the startle reflex methodology provides a unique tool to more directly link animal and human research, since inference about aversive states is made using the same measure in the two species. Finally, aversive conditioning provides a framework to study cognitive and emotional interactions during the processing of threat information. Indeed, aversive events usually do not happen unexpectedly. Their occurrence may be predicted based on prior experience. Associative learning mechanisms, such as classical fear conditioning, are central to the development of appropriate expectancies about upcoming events.

The present article will first review findings from startle studies in animals and in humans that suggest the existence of at least two distinct defense mechanisms, one activated by explicit cues and the other by threatening contexts. Studies will then be presented that provide emerging evidence that these defense systems are differentially activated in anxious individuals or in patients with anxiety disorders compared to healthy subjects. It will be shown that in threatening contexts, anxious patients generate aversive responses in a less discriminating manner compared to nonanxious subjects. It will be argued that these aversive responses resemble symptoms in animals during context conditioning. Finally, the possible role of factors that affect associative learning and conditioned responses in the etiology and maintenance of anxiety and anxiety disorders will be discussed.

Apprehension/General Distress versus Explicit Cued Fear

Clinicians have long recognized that anxiety is not a unitary phenomenon and that it can take several forms (Barlow 2000; Kandel 1983). The DSM-IV identifies several specific anxiety disorders, suggesting heterogeneity of symptoms and etiology among them (American Psychiatric Association 1994). Anxiety disorders are thought to result from abnormal processing of threat-related stimuli (Beck and Clark 1997; Eysenck 1991), as well as functional deficits in brain pathways underlying fear learning and memory (Barlow 2000; Rosen and Schulkin 1998). Despite symptom heterogeneity, a strong case can be made for differentiating between at least two aversive states, namely fear and anxiety (Marks 1969). Fear is a normal response to a realistic and imminent danger. On the other hand, anxiety is not linked to an objective source of danger and is more future-oriented (Marks 1969). Fear is usually viewed as a phasic response associated with a predominantly well-defined and identifiable threatening stimulus, whereas anxiety is more sustained and generalized and is not restricted or linked to a specific cue (i.e., it is free-floating). Thus, from an experimental standpoint, two characteristics may distinguish stimuli or situations that elicit fear versus anxiety: the source of danger and its timing or predictability (imminent vs. future-oriented). In addition, it is probable that the intensity of the aversive response distinguishes fear from anxiety, with anxiety being less intense than fear.

Clinically, fear is equivalent to phobic anxiety in that it is an emotional alarm system specifically attuned to preserve the well-being of the individual from potentially life-threatening situations (Barlow 1988). By contrast, anxiety is induced by the perception of insecurity. It can be conceptualized as an emotional system activated by uncertainty and by the expectation of adversity (Rosen and Schulkin 1998) that can lead to excessive worry, strong somatic and physiologic signs of arousal, increased vigilance, behavioral avoidance, and significant impairment in functioning (Barlow 2000; Kandel 1983). Central to generalized anxiety are feelings of helplessness, a perceived sense of unpredictability, and the anticipation of potential future aversive events (Barlow 2000; Mineka and Zinbarg 1996).

Pavlovian Conditioning

The distinction between qualitatively different fear/anxiety systems is consistent with findings from animal models (Blanchard et al 1993; Davis 1998; File et al 1998; File et al 1999). The assumption underlying most animal models of anxiety disorders is that anxiety evolves from defense mechanisms essential for survival, which are highly conserved across species (LeDoux 1995; Rodgers 1997). According to this view, symptoms of pathologic anxiety (hypervigilance, anxious anticipation, avoidance, escape, exaggerated startle, and somatic and autonomic symptoms) are the result of inappropriate activation of normally adaptive defense systems. Because anxiety disorders can be conceptualized as disorders of defense mechanisms activated inappropriately, the vast amount of animal data on psychobiological mechanisms underlying the detection of threat provides a rich source of information to guide human research.

Pavlovian aversive conditioned responses present striking resemblance with the symptoms of fear and anxiety. During aversive conditioning, animals receive a mildly aversive unconditioned stimulus (US) (e.g., a shock) that is repeatedly paired with a neutral conditioned stimulus (CS) (e.g., a light). Subsequently, these animals will exhibit symptoms of fear and anxiety (e.g., freezing response, increased startle) to both the neutral CS and the experimental context (i.e., cage) in which the shock was administered. The learned fear responses to the CS and to the training environment are referred to as explicit cue and

context conditioning, respectively. *Cued fear* conditioning has been viewed as a model of fear and fear-related disorders, such as phobias (Ohman and Mineka 2001). During cued fear conditioning, the animals learn to fear a clear threat signal (CS) that predicts an imminent danger. The presentation of the threat signal induces a brief period of fear that subsides shortly after the offset of threat; however, cued fear conditioning does not model the essential features of anxiety, which is activated in a less discriminant way and focuses on future potential threat. The hypervigilance and persistent signs of generalized distress that characterize anxiety may be better modeled by context conditioning, since anxiety is neither triggered nor suppressed by an explicit cue.

Substantial evidence from neurobiological studies points to the role of different neural systems in cued fear and context conditioning (Davis 1998; LeDoux 1998). In particular, the various nuclei of the amygdala are responsible for the acquisition and expression of cued fear conditioning (Davis 1998; LeDoux 1998). Other structures, along with the amygdala, have been implicated in context conditioning. Several studies indicate that the dorsal portion of the hippocampus plays a pivotal role in context conditioning (Kim and Fanselow 1992; Phillips and LeDoux 1992). Whether the hippocampus, a structure that has also been associated with mood and anxiety disorders (Sapolsky 2000), is necessary for context conditioning is currently a matter of debate (McNish et al 1997). The conclusion that the hippocampus is involved in context conditioning is based on the finding that lesions of the dorsal hippocampus attenuate freezing to contextual cues (Kim and Fanselow 1992; Phillips and LeDoux 1992). At issue is the extent to which lesions of the dorsal hippocampus interfere with the expression of the freezing response per se, rather than with contextual memories (McNish et al 1997). Another issue is whether the dorsal hippocampus is only necessary for the mnemonic operations necessary to encode the spatial features of the context or affect anxiety states independent of its role in memory. Below, we will review findings derived from startle studies that suggest that another structure, the bed nucleus of the stria terminalis, may underlie the stress associated with exposure to threatening contexts (Davis 1998).

The Startle Reflex and Aversive States

The central nucleus of the amygdala has extensive connections to hypothalamic and brainstem sites responsible for the physiologic signs and behavioral symptoms of fear (Davis 2000). In humans, these physiologic responses can be measured using psychophysiological techniques. Most of our knowledge on fear conditioning in humans is derived from investigations of electrodermal activity and, to a lesser degree, of cardiovascular activity. A relatively new measure, the startle reflex, may be the most valid investigative tool to explore aversive emotional responses (see below).

The startle reflex is a response to an intense and surprising stimulus. In animals, startle is measured by assessing the whole body reflex. In humans, the "startle pattern" consists of a forward thrusting of the head and a descending flexor wave reaction, extending through the trunk and the knees (Landis and Hunt 1939). The amplitude and the latency of the startle reflex can be measured by recording the eyeblink reflex, the most consistent and persistent component of the startle pattern. Although a startle response can be elicited with visual and tactile stimuli as long as the stimuli are sufficiently intense and have a fast rise time, most startle studies use acoustic stimuli. A typical acoustic startle is a brief (e.g., 40 millisecond) burst of white noise with an abrupt onset and an intensity ranging from 90 to 115 A-weighted decibels dBA.

Fear-Potentiated Startle

Fear-potentiated startle refers to the increase or potentiation of the startle reflex during fear states elicited by the anticipation of an aversive stimulus (e.g., a shock). This effect was first described in animals using aversive conditioning procedures by Brown et al (1951) and has been investigated extensively by Davis and his collaborators (Davis 1998). In a typical experiment, the amplitude of the startle reflex elicited by a startling stimulus (e.g., loud noise) is measured either in the presence or in the absence of a CS previously paired with an aversive US. Under these conditions, the amplitude of the startle reflex is greater in the presence of the CS than in the absence of the CS. *Fear-potentiated startle*, defined as the increase in startle amplitude to startle stimuli delivered during the CS compared to startle stimuli delivered in the absence of the CS (e.g., during intertrial interval), is considered an operational measure of fear. The same effect has been found in humans (Grillon and Davis 1997; Hamm et al 1993; Lipp et al 1993; Spence and Norris 1950). One of the advantages of the fear-potentiated startle procedure to study aversive responses is that very similar procedures potentiate startle in animals and in humans (see below). The possibility of replicating animal findings in humans (or vice versa) enhances the cross-fertilization between psychological sciences and neurosciences and validates animal models of human behaviors. Because the neural circuitry responsible for the potentiation of startle by aversive states is fairly well understood in animals, the startle reflex methodology enables human research to take

advantage of advanced knowledge about neurobiological mechanisms of defense behaviors in animals.

Another advantage of startle is that it is under control of the experimenters. In a typical fear-potentiated startle experiment, startle-evoking stimuli can be presented at any given time, functioning as a *probe* of changes in emotions and thus providing an index of ongoing affective information processing, regardless of the presence or absence of a discrete emotional cue (e.g., a CS or an emotional picture). Such an approach is virtually impossible with the most traditional psychophysiological measures of fear conditioning, such as the skin conductance response (SCR). Indeed, the SCR is by definition a response to a stimulus. It provides a measure of autonomic arousal in response to the discrete emotional cue. In contrast, startle can track rapid changes in emotional states prior, during, and after the presentation of an emotional stimulus in a way that the electrodermal system cannot match. This is particularly important for fear conditioning studies where comparisons in emotional reactivity to the CS and to the context can be assessed with the startle reflex but not with the skin conductance.

Davis and his collaborators have conducted extensive studies delineating the neural circuit involved in startle potentiation by explicit conditioned cues (Hitchcock and Davis, 1986; Hitchcock and Davis 1991; Rosen et al 1991). A short-latency primary pathway is responsible for the elicitation of the startle response. In the rat, this primary acoustic startle pathway consists of only three synapses (cochlear root neurons, neurons in the nucleus reticularis pontis caudalis, and motoneurons in the spinal cord) (Lee et al 1996). A secondary modulatory pathway involving the amygdala is responsible for the potentiation of startle by explicit threat cues. The central nucleus of the amygdala projects directly to the startle pathway, at the level of the nucleus reticularis pontis caudalis. Lesions of the amygdala or at several points on the connection between the amygdala and the nucleus reticularis pontis caudalis block the expression of fear-potentiated startle to conditioned cues (Campeau and Davis 1995; Hitchcock and Davis 1986; Hitchcock and Davis 1991).

Studies in humans using threat of shock procedures have confirmed the crucial role of the amygdala in startle potentiation, further validating the fear-potentiated startle methodology as a measure of fear. During threat of shock, subjects are verbally informed that shocks can be administered in the presence of *threat* signals but not during safe signals. Threat of shock procedures yield highly reliable and robust startle potentiation (Grillon et al 1991). Funayama et al (2001) found that patients with unilateral left temporal lobectomy including the amygdala failed to show potentiated startle during threat of shock. This was in contrast to the robust startle potentiation observed in patients with unilateral right temporal lobectomy or individuals with no brain lesions. This lateralization is consistent with previous threat of shock studies (Grillon and Davis 1995; Phelps et al 2001). Using monaural stimulation, Grillon and Davis (1995) reported greater startle potentiation to startle stimuli delivered to the right ear/left hemisphere compared to left ear/right hemisphere. Additional evidence of left amygdala involvement was reported in a functional magnetic resonance imaging (fMRI) study during a verbal threat procedure (Phelps et al 2001). A full discussion of these laterality results is beyond the aim of this review. It suffices to say that the amygdala is critically involved in human fear-potentiated startle. The greater involvement of the left versus right amygdala may reflect the way threat is communicated in threat experiments. The left hemisphere may be more engaged when subjects learn the aversive nature of the threat stimulus through verbal communications (Phelps et al 2001).

The Neurobiology of Contextual Fear

Recent evidence from animal studies in Davis' laboratory indicates that the startle reflex is potentiated in conditions that are reminiscent of generalized anxiety rather than fear. For example, under certain conditions, baseline startle reflex shows a gradual elevation over the course of aversive conditioning that may reflect a response to chronic stress (Gewirtz et al 1998). This elevation is blocked by lesions of the bed nucleus of the stria terminalis but not by lesions of the amygdala (Gewirtz et al 1998). The BNST is very similar to the amygdala in terms of morphology, transmitter contents, and efferents (Alheid et al 1995), but it seems to play a different role than the amygdala in the modulation of aversive responses. For example, unlike lesions of the amygdala, lesions of the BNST do not block cued fear-potentiated startle (Walker and Davis 1997). Further evidence of a functional dissociation between the amygdala and the BNST is suggested by the fact that lesions of the BNST, but not lesions of the amygdala, block the so-called light-enhanced startle, which is the enhancement of startle in animals exposed to prolonged bright lights (Walker and Davis 1997). The stress hormone corticotropin-releasing hormone (CRH) may be involved in the potentiation of startle by chronic stress and anxiety. It is now well established that CRH has anxiogenic properties. The CRH antagonist α-helical CRH9-41 suppresses the behavioral and neuroendocrine effect of stressors (Dunn and Berridge 1990). In addition, intraventricular administration of CRH produces a long-lasting potentiation of the startle reflex (Liang et al 1992; Swerdlow et al 1986), which is suppressed by the anxiolytic benzodiazepine chlordiazepoxide (Swerdlow et al 1986). Davis and his collaborators have shown that the

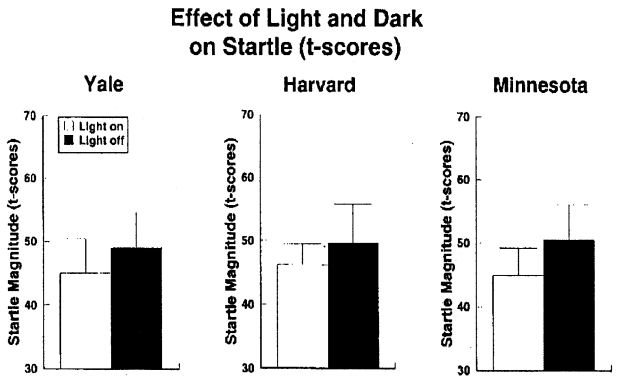

Figure 1. Effect of darkness on the magnitude of the startle reflex in children and adolescents. Acoustic startle stimuli were delivered in an illuminated room (light on) or in complete darkness (light off). Results of a collaborative study involving Yale University, Harvard University, and the University of Minnesota. (Reproduced with permission from Grillon et al 1999.)

BNST, not the amygdala, is responsible for the sustained potentiation of startle by CRH (Lee and Davis 1997). These results suggest that the symptom of aversive anticipation that characterizes anxiety may be mediated by a sustained activation of the BNST via corticotropin releasing hormone (Davis 1998).

Contextual Fear in Humans

The startle reflex is also highly sensitive to contextual stressors in humans. During fear conditioning procedures, various contextual cues can potentiate startle. For example, startle is affected by the aversive nature of the experiment itself. Thus, baseline startle is greater before a fear conditioning experiment during which shocks are administered, compared to an experiment where there is no aversive stimulus (Bocker et al 2001). The shock electrodes are also potent contextual cues, producing anxiety and further increasing startle, even when there is no imminent risk of a shock (Grillon and Ameli 1998). This contextual sensitization possibly reflects sustained apprehensive anticipation and worries about the future administration of the shock. Because of this anxious apprehension, there is no neutral baseline startle level during fear conditioning experiments; the fear-potentiation of startle by an explicit threat cue is riding on an already elevated baseline level reflecting contextual fear.

Anxiogenic situations such as darkness also facilitate startle (Grillon et al 1997b). Startle is greater when elicited in complete darkness compared to an illuminated room, both in children (Figure 1) (Grillon et al 1999) and in adults. We have suggested that the light-enhanced startle in rats and the facilitation of startle in the dark have similar evolutionary bases (Grillon et al 1997b). Rats are nocturnal animals and are vulnerable in bright spaces, whereas humans are diurnal and are more vulnerable in the dark. In general, threatening environments facilitate startle in both species.

Contextual Fear and Anxiety Disorders

Recently, we have reported that darkness is also a powerful aversive stimulus in Vietnam veterans with posttraumatic stress disorder (PTSD). PTSD veterans show an exaggerated facilitation of startle in the dark (Grillon et al 1998b). Poor sleep and fear of the dark are frequent symptoms in hypervigilant Vietnam veterans with PTSD. The onset of darkness signals a period of anxious anticipation, a scanning of the environment for potential dangers, and an inability to feel safe. As one veteran describes, “The only time I really sleep is at daybreak. I hate darkness and the night. I just can’t stay asleep. Every noise bothers me. And even if I do sleep, it’s like sleeping with an eye open. When morning comes I can relax and go to sleep” (Grillon et al 1998b). These feelings may be viewed as conditioned emotional responses to an explicit cue, darkness, which serves to potentiate startle. Alternatively, PTSD veterans may be overly sensitive to threatening contexts. Generalized aversive anticipation in the face of perceived threats may be intensified by darkness in PTSD, a phenomenon that is reminiscent of the aversive response of rodents exposed to bright lights. In general, our results in individuals with PTSD, as well as with other anxiety disorders, are in agreement with this hypothesis, suggesting greater sensitivity to contextual cues in patients compared to controls.

Table 1. Baseline and Fear-Potentiated Startle in Patients with Anxiety Disorders

Authors	Experimental groups	Procedure	Potentiated startle	Baseline startle
Grillon et al (1994)	Panic disorder	Verbal threat	Normal	Elevated
Cuthbert et al (1994)	Various anxiety disorders	Emotional imagery	Normal	Elevated in PTSD and panic disorder
Morgan et al (1995)	PTSD	Verbal threat	Normal	Elevated
Grillon et al (1998c)	PTSD	Verbal threat	Normal	Elevated
Kumari et al (2001)	OCD	Affective pictures	Normal	Elevated

OCD, obsessive-compulsive disorder; PTSD, posttraumatic stress disorder.

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Table 1 lists a series of startle studies in patients with anxiety disorders in studies using various procedures to potentiate startle (e.g., threat of shock, affective pictures). In all these studies, baseline startle levels were increased throughout the experiment in the patients compared to the controls. Possibly, this effect is prompted by the threatening experimental context, which is perceived by the patients as abnormally stressful. It is likely that the ambient stress of experimental settings potentiate startle in a way analogous to that observed in an animal that is returned to an aversive context where it has previously received shocks. Unlike animals, people do not necessarily need to be conditioned to fear specific situations. Human ability for abstract thoughts enables them to anticipate aversive events based on verbal communications.

The contextual fear hypothesis could explain the sustained elevation of baseline startle in anxious patients in aversive contexts; however, there are alternative explanations. One possibility is that individuals who eventually develop anxiety disorders have generally higher levels of startle before the onset of the disorder. In fact, children who are at high risk for anxiety disorders because of a parental history for these conditions exhibit greater baseline startle responses compared to low-risk children (see below). Again, this could also be attributed to the stress of the experimental setting in which loud and unpleasant startling stimuli are delivered (Grillon et al 1997a). Another possibility is that the elevation of startle in anxious patients results from a persistent sensitization caused by chronic arousal secondary to a traumatic incident. There are, however, very few animal data that would support *long-duration* and sustained sensitization of startle. In fact, Davis (1989) reviewed evidence suggesting that the relationship between stress and startle sensitization was not consistent. In rats, prior stress either has little effect or can reduce subsequent startle. On the other hand, a brief electric shock can substantially increase startle in rats (Davis 1989; Krase et al 1994) and in humans (Hamm and Stark 1993), although this effect only seems to last for an hour or so. Stronger, more pronounced shocks can increase startle for up to 4 days but not 10 days (Servatius et al 1994). Thus far, persistent sensitization of startle has been difficult to demonstrate, although this effect could theoretically explain the elevation of startle in anxious patients.

To demonstrate that the increase in baseline startle in anxious patients is a transient effect caused by aversive contexts rather than a persistent trait variable or a proclivity to long-term sensitization, we conducted a study in which Vietnam veterans with PTSD were tested on two separate occasions that contained varying degrees of ambient stress (Grillon et al 1998c). During the first session, only startle stimuli were delivered, yielding a low

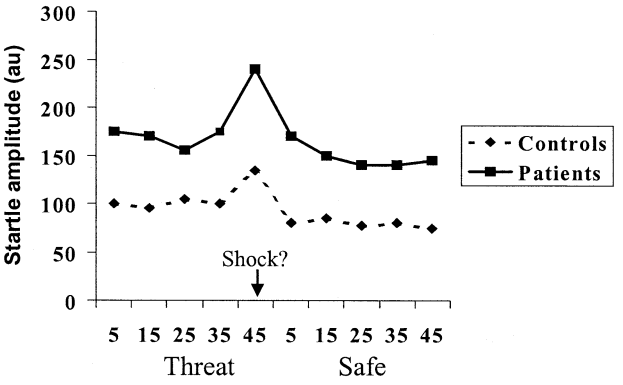

Figure 2. Fear-potentiated startle to threat of shock in patients with panic disorders and healthy controls. Subjects were informed that shocks could be administered only during the last 10 seconds of a threat condition signaled by a geometric shape on a computer (see text for additional information concerning the experiment). (Adapted and reproduced with permission from Grillon et al 1994.)

level of stress. In contrast, the ambient stress level was considerably increased in the second session by conducting a threat of shock experiment. As predicted, baseline startle did not differ among patients and controls in the low-stress environment of the first session. In contrast, in the more stressful second session, the PTSD veterans exhibited a significantly exaggerated startle response that was further elevated following the placement of the shock electrodes (Grillon et al 1998c).

Anxious patients exhibit exaggerated anxiety in threatening contexts, but they do not show enhanced fear to explicit threat signals when the threat is verbally mediated. Grillon et al (1994) reported normal fear-potentiated startle in patients with panic disorder in a study that examined the time course of fear (Figure 2). In this study, a shock could only be delivered at the end of a 50-second duration threat signal. Subjects were told to expect a shock in the last 10 seconds of the threat condition. A clock that counted time down from 50 to 0 seconds was activated with the onset of the threat signal. Acoustic startle probes were administered at different times during and in the absence of the threat signal to assess changes in emotional states. Results showed a small startle potentiation following the onset of the threat signal. This was followed by a large startle potentiation at the time of shock expectancy and return to baseline levels within 5 seconds of threat signal offset. As Figure 2 indicates, baseline startle was enhanced in the patients, compared to the controls; however, the pattern of startle potentiation to the threat signal did not differ among patients and controls, suggesting similar timing of affective responses to the explicit threat cue in the two groups. Similar results were obtained with Vietnam veterans with PTSD (Morgan et al 1995).

Much work needs to be done to characterize the potentiation of startle to explicit and contextual cues in patients with anxiety disorders. Among important issues to be addressed is the nature of contextual cues. Stimuli or situations that lead to contextual fear in humans and animals are likely to differ greatly. Animals learn to associate contextual cues with the aversive stimulus during conditioning. In humans participating in a fear conditioning experiment, some of this association is already present upon arrival in the laboratory because of the participants' knowledge of the aversive nature of the experiment following their recruitment and informed consent. Hence, instructions are powerful contextual cues that can greatly influence baseline startle. Shock electrodes are also considered contextual cues rather than explicit cues for shocks because they do not provide precise information about the time of shock administration. The aversive response they engender is future-oriented. For example, animals conditioned with a long trace interval (e.g., a shock administered several seconds after the offset of the CS) will show more contextual fear than when trained in a delay conditioning procedure (shock delivered at CS offset) (Marlin 1981). When the shock signaled by a CS is remote in time, it becomes more difficult to predict. As a result, the contextual cues become the best predictor of shocks.

Despite these issues regarding the nature of the contextual stimuli, published results are highly suggestive of the crucial role of contextual fear in human anxiety. Clinically anxious patients appear overly sensitive to stressful contexts, but they do not show exaggerated fear responses to explicit cues. This pattern of affective modulation, possibly a distinctive characteristic of anxiety disorders, may reflect hyperexcitability of neural structures underlying contextual fear or chronic stress, including the bed nucleus of the stria terminalis. An exception to this finding is patients who suffer from fear disorders, such as simple phobias. Not surprisingly, these patients exhibit exaggerated startle responses when confronted with their feared object. Vrana et al (1992) showed a transient exaggeration of startle reflex in one bird phobic individual using imagery procedure. Similarly, relative to control subjects, spider phobics show exaggerated startle when viewing pictures of spiders (de Jong et al 1996). These results further suggest a qualitative distinction between explicit and contextual cues.

Potentiated Startle and Risks for Anxiety Disorders

All major subtypes of anxiety disorders aggregate in families (Merikangas et al 1998). The investigation of offspring of parents with these conditions is, therefore, a powerful strategy to identify premorbid risk and protective factors, as well as early signs of expression of these disorders. The finding of increased baseline startle reactivity in individuals with PTSD and panic disorder raises the possibility that a proclivity to react fearfully to situations that are only mildly challenging to others is a risk marker for subsequent development of anxiety disorders. We reported results that are consistent with this hypothesis (Grillon et al 1997a; Grillon et al 1998a). Grillon et al (1998a) examined fear-potentiated startle to threat in children of parents with an anxiety disorder. Because shocks may be more difficult to use with children than with adults, we developed a procedure in which a strong puff of air (airblast) delivered to the neck was substituted for the shock. Anticipation of such an airblast is mildly anxiogenic. It produces a robust startle potentiation in children (Grillon et al 1999) and is associated with increased activity in the amygdala based on fMRI studies in adults (Pine et al 2001).

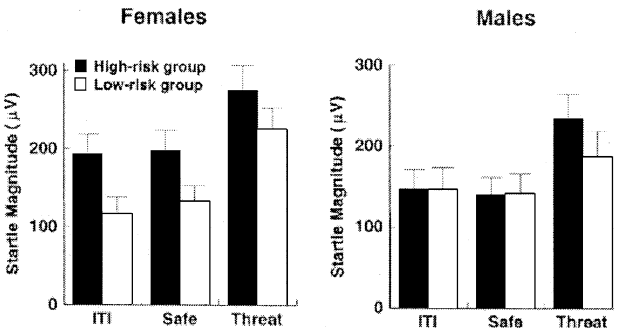

Figure 3. Fear-potentiated startle to threat of airblast in boys and girls at risk for anxiety disorders. The subjects were children and adolescent offspring of parents with (high-risk group) or without (low-risk group) an anxiety disorder. Subjects were told that a strong puff of air (directed to the neck at the level of the larynx) would be delivered during threat but not during safe conditions. Twelve-second duration lights of different colors signaled the safe and the threat conditions. Startle stimuli were delivered in the presence and in the absence (intertrial interval or ITI) of the lights. (Reproduced with permission from Grillon et al 1998a.)

The findings pointed to gender-specific abnormal startle reactivity in the high-risk group. High-risk girls were overly sensitive to contextual threat but exhibited normal fear-potentiated startle to explicit threat, and boys showed the inverse pattern (normal contextual fear and increased fear potentiation to threat) (Figure 3). These results suggest that vulnerability to anxiety disorders may involve a gender-specific differential sensitivity of fear pathways. In light of the well-documented propensity of females to be affected by mood and anxiety disorders compared to males (Kessler et al 1994), these results should provide an impetus to study gender differences in activity of the BNST in animal models.

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The Psychopharmacology of Fear-Potentiated Startle

Theoretically, support for the existence of separate defense mechanisms could be bolstered by the demonstration that anxiolytic drugs have a differential effect on fear and anxiety. For example, given that benzodiazepines relieve anxiety but are not particularly efficient in the treatment of phobias (Marks 1987) for which exposure therapy is advised, one could expect that benzodiazepines reduce contextual fear but have little effect on cued fear. In support of such a possibility, Blanchard et al (1993) reviewed evidence that benzodiazepines had little effect on *fear* responses in the Fear/Defense Test battery but reduced *anxiety* behaviors significantly in the Anxiety/Defense Test battery.

There are currently few published psychopharmacology studies of fear-potentiated startle in humans. The bulk of the research with anxiolytic drugs in animals concerns the benzodiazepines. In rats, benzodiazepines reduce fear-potentiated startle to explicit conditioned cues (Davis 1979; Hijzen and Slangen 1989; Hijzen et al 1995; Joordens et al 1998; Guscott et al 2000), but there are no clear-cut results in humans. Two human studies reported a reduction of fear-potentiated startle to a threat cue following administration of diazepam (Bitsios et al 1999) or alprazolam (Riba et al 2001); however, a different conclusion was reached by Riba et al (1999) using lorazepam and by Baas et al (2002) in a collaborative investigation at two different sites (Utrecht University and Yale university) that reported the results of four separate experiments using diazepam and oxazepam. In this latter study, both diazepam and oxazepam reduced baseline startle reactivity, but these drugs did not affect fear-potentiated startle to a threat cue (Figure 4). An effect of benzodiazepines on human contextual fear was suggested by the finding that diazepam suppressed the facilitation of startle by darkness. In addition, multiple regression analyses indicated that the benzodiazepine-induced reduction in baseline startle was not only due to sedation but also to the anxiolytic effect of the treatment on contextual fear. A similar conclusion was reached by Guscott et al (2000) in a study in rats.

The reason for the discrepancies between studies is unclear. Procedural differences could play a role (Baas et al 2002). For example, in the Bitsios et al (1999) study, the shock electrodes were removed during the safe condition and reattached during the threat condition. Hence, assuming shock electrodes are contextual stimuli, there was a confounding of explicit and contextual cues. A possible explanation for the lack of effect of benzodiazepines on fear-potentiated startle in the Baas et al (2002) study is that the startle response was at a ceiling level during the threat of shock. Hence, reduction in fear was not reflected in a similar reduction in startle amplitude; however, a critical

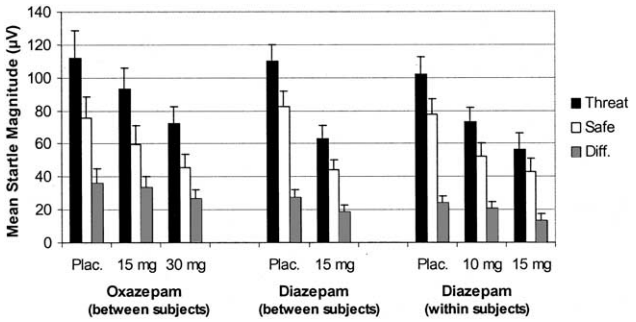

Figure 4. Effect of two benzodiazepines on fear-potentiated startle to a threat cue. Subjects were told to expect shocks in the threat (Threat) but not in the safe (Safe) condition. Effects of 15 mg and 30 mg of oxazepam (left panel) and 15 mg of diazepam (middle panel) in between-subject designs. Effect of diazepam in a within-subject design (right panel). Subjects were tested in three separate sessions on different days. On each session, they received placebo, 15 mg diazepam, or 30 mg diazepam. The benzodiazepines reduced the overall startle reactivity but did not affect fear-potentiated startle expressed as a difference score (Diff) or as a percent score (not shown). (Adapted and reproduced with permission from Baas et al 2002.)

issue that plagues research using the startle reflex is the manner in which fear-potentiated startle is calculated. Fear-potentiated startle is usually calculated as a difference score between the amplitude of startle during a control baseline condition and the amplitude of startle during the threat condition. Difference scores are appropriate when baseline startle amplitude is unchanged across experimental conditions (e.g., drug treatments); however, because benzodiazepines greatly reduce overall startle reactivity, this approach may be inappropriate due to the fact that the absolute magnitude of startle potentiation is affected by changes in baseline reactivity. Recent animal data support this idea. Walker and Davis (in press) injected strychnine, a glycine receptor antagonist that increases baseline startle amplitude nonspecifically. Strychnine was found to increase significantly the absolute, but not the percent, fear-potentiated startle score. It is likely that a drug that nonspecifically reduces startle reactivity works in a similar manner. Hence, standard scores or ratio scores would be a more appropriate choice to analyze fear-potentiated startle in studies testing drugs that affect baseline startle.

A lack of effect of benzodiazepines on fear-potentiated startle would be contrary to results in animals; however, if explicit *cued threat* models fear, the failure to find a reduction of startle with benzodiazepines in humans is consistent with the clinical evidence that benzodiazepines are relatively ineffective in the treatment of fear/phobic-related disorders but alleviate symptoms of generalized anxiety (Marks 1987). In addition, the criticism concern-

ing measurement of baseline startle in humans could also apply to animal studies. Further, it is not clear how doses administered to animals compare to the clinical doses in humans. On the other hand, relatively low doses of benzodiazepines that have minimal impact on baseline startle reduce fear-potentiated startle (Davis 1979; Vale and Green 1996). Finally, animal studies use classical conditioning procedures, whereas all the human studies have been conducted with instructed threat. A threat experiment tests only the expression of fear, whereas in a conditioning experiment, both the expression of fear and the memory for the CS-US association are examined. Given that benzodiazepines impair memory processes and impede cognitive and attentional processing of a broad range of stimuli (Sarter et al 1994), it is possible that benzodiazepines prevent the retrieval of the CS-US association in conditioning experiments.

To conclude this section, it should be kept in mind that the psychopharmacology of human fear-potentiated startle is currently at its infancy, and no clear conclusions can be drawn. There are a number of issues that need to be resolved to enhance our understanding of psychopharmacology of fear-potentiated startle, including the role of sedation versus anxiolysis, the effects of the drug on attention and memory processes, and the quantification of fear-potentiated startle with respect to control values.

Classical Conditioning, Contextual Fear, and Sensitization

The literature on the effects of unpredictable and uncontrollable aversive events provides important insights into features of anxiety disorders associated with contextual fear. Predictability and controllability are central to several models of anxiety (Barlow 2000; Foa et al 1992; Mineka and Kihlstrom 1978). Barlow (2000) talks about *anxious apprehension* as a state of nervous helplessness due to a perceived inability to predict or control upcoming events. Barlow's model of anxiety is based on the similarities between anxiety symptoms and the disturbances observed in animals exposed to unpredictable or uncontrollable shocks. In animals, unpredictable aversive stimuli produce debilitating cognitive, behavioral, and somatic effects that are not obtained when the aversive stimuli are predictable (Maier 1991; Mineka and Kihlstrom 1978).

Predictability, which can be defined as the extent to which an event is signaled reliably, determines the nature of aversive conditioned response (Fanselow 1980; Marlin 1981; Odling-Smee 1975). Signaled shocks following conditioning lead to cued fear, while nonsignaled shocks lead to contextual fear. The enhancement of contextual fear when shocks are unpredictable is consistent with conditioning theories (Rescorla and Wagner 1972). Seligman's safety-signal hypothesis provides a clear explanation for the effects of unpredictability (Seligman and Binik 1977). The basic tenet of the safety-signal hypothesis is that when an organism can predict threat because it is signaled, the absence of signal for threat also predicts the absence of danger, that is, safety; however, when shocks are not signaled, periods of safety are also not signaled, and the organism remains in a state of chronic anxiety.

The increase in contextual fear to unpredictable shock provides an analogy for the increased startle observed in anxious patients; however, the literature on *context conditioning* in humans is virtually nonexistent, raising questions as to the applicability of this model to humans. To address this question, we conducted a study where shocks were administered predictably and unpredictably (Grillon and Davis 1997). The study involved three groups of subjects participating in a single-cue fear conditioning procedure over two experimental sessions, designed to assess both cued and context conditioning. In a *paired* group, a shock was delivered at the offset of the CS. In an *unpaired* group, a shock was randomly administered in the absence of the CS. Thus, the shock was administered predictably in the paired group and unpredictably in the unpaired group. Context conditioning was assessed 4–5 days later, when subjects returned to the conditioning context. A control group participated in a nonaversive conditioning experiment in which a signal for button press (reaction time task) was activated at the offset of the CS. This group served as a control for long-term habituation of startle over two experimental sessions. Acoustic startle probes were presented before conditioning and during conditioning in the presence of the CS and in its absence (i.e., during intertrial interval [ITI]). Startle responses before conditioning provided an assessment of contextual fear before shock administration. Startle responses during conditioning provided an assessment of conditioned performance to the CS. As expected, successful conditioning to the explicit CS was obtained in the paired group but not in the unpaired group during the first session. This was reflected in the greater startle potentiation to the CS compared to ITI during acquisition in the paired group only. In addition, initial unreinforced (no shock administered) presentations of the CS during the second session led to startle potentiation to the CS relative to ITI in the paired group only, indicating that conditioning performance was well retained in this group.

Crucially, comparison of baseline startle obtained before the actual conditioning phase in each session showed greater responses in session 2 compared to session 1 in the unpaired group. This contrasted with results in the paired group, which showed no changes in baseline startle between the two sessions. Consistent with conditioning theories (Rescorla and Wagner 1972), paired presentation

of an explicit CS and a shock results in conditioned fear to the explicit CS and little contextual fear, whereas unpaired presentation of the CS and the shock leads to no explicit *cue* conditioning but significant context conditioning.

The results in the unpaired group are analogous to the findings in anxious patients. Both groups show elevated baseline startle levels in a threatening experimental context, even in the absence of imminent risk. Similar mechanisms may be responsible for the enhanced anxiety in the unpaired group and in the anxious patients. In the anxious patients, the exaggerated startle response reflected an *anxious apprehension* caused by a perceived inability to predict upcoming threats. Unpredictability was also a causal factor in the abnormal responses in the paired group; however, this sense of unpredictability was based on appropriate learning, that is, with a previous experience with nonsignaled shocks.

Context Conditioning in Veterans with PTSD

Most evidence showing enhanced contextual fear in patients with anxiety disorders is derived from threat of shock paradigms, where information concerning danger is verbally mediated. Recently, we have investigated contextual fear using *conditioning* in Gulf war veterans with PTSD (Grillon and Morgan 1999). In this study, a differential conditioning procedure was used. During differential fear conditioning, only one of two stimuli, the CS+, is paired with a shock. Conditioned fear usually develops to the CS+ but not to the other stimulus, the CS−. Based on our previous findings, we predicted that the PTSD veterans would show greater context conditioning when returning to the conditioning context, compared to the non-PTSD veterans.

Results were consistent with our prediction (Figure 5). Relative to the non-PTSD veterans, baseline startle in the PTSD veterans was increased from the initial conditioning session to the second session.[1] This effect was significant even before the placement of the shock electrodes, when subjects were clearly not at risk of receiving a shock. The findings in the PTSD veterans are remarkably similar to that of healthy subjects who received unpaired CS shock (Grillon and Davis 1997). Collectively, these results further emphasize the pivotal role of shock unpredictability on contextual fear and, by extension, on anxiety symptoms.

[1] The veterans with PTSD showed larger startle responses compared to the non-PTSD veterans in the first session; however, the data of two non-PTSD veterans were excluded from the analysis because they exhibited virtually no eyeblink responses to the startle stimuli. In addition, results of a PTSD veteran, who had the largest startle response in the first session, were also excluded from the analysis because he did not return for the second session.

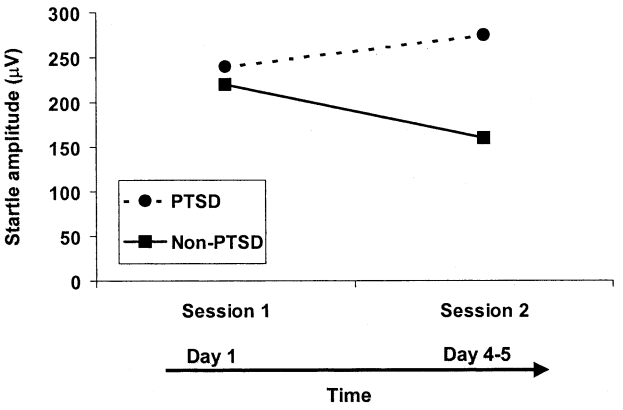

Figure 5. Context conditioning in Gulf war veterans with and without PTSD. Subjects participated in two conditioning sessions separated by a few days. Baseline startle amplitude to acoustic startle stimuli presented before differential aversive conditioning on each of two sessions separated by 4–5 days in Gulf war veterans with and without PTSD. Baseline startle did not differ between the two groups during the first session but was significantly larger in the PTSD compared to the non-PTSD veterans upon returning in the experimental room. (Adapted and reproduced with permission from Grillon and Morgan 1999.)

Associative Learning Deficits and Contextual Fear

Unpredictable aversive events increase anxious apprehension, but most of the events in our surroundings are not necessarily unpredictable or unexpected. Associative learning and conditioning are mechanisms by which organisms derive predictability, reduce uncertainty, and learn to predict events in the environment. An important development in the learning theory literature in recent years is the acknowledgment that conditioning is not a low-level reflexive stimulus-response process but a highly complex cognitive operation. Contemporary models, inspired by Rescorla and Wagner (1972), view conditioning as an adaptive cognitive process that enables organisms to develop expectancies and learn to anticipate events, aversive or otherwise (Mineka and Zinbarg 1996). In humans, this process is dependent on the formation of CS-US associations that rely on controlled processing indexed by verbal awareness of the CS-US contingency and by the development of a skin conductance response (Dawson and Furedy 1976; Purkis and Lipp 2001). There may be an exception to this cognitive interpretation of conditioning. Certain "prepared" stimuli (e.g., spiders, snakes) can enter into associations via conditioning processes that take place largely outside of one's awareness (Ohman and Mineka 2001) (but see Lovibond and Shanks 2002).

Conditioning is an adaptive process. One such adaptive function is that it reduces uncertainty, which may be valued for its own sake. In aversive situations, condition-

ing reduces the range of stimuli that elicit fear. During aversive conditioning, the initial presentation of the shock is unpredictable, and fear generalizes to all the surrounding contextual cues. Following several CS-shock pairings, this contextual sensitization becomes stimulus-specific and fear to the context is inhibited (Pavlov 1927). Thus, during fear conditioning, organisms learn to gradually identify the CS as the danger signal and the context as a safety signal. As a result, the CS, which elicits a transient fear response, reduced overall contextual anxiety; however, a *signaled* shock is not necessarily a *predictable* shock. The shock becomes predictable following successful associative learning. In fact, there is a large interindividual variability in conditioning performance in humans. While some subjects acquire cued fear conditioning, others do not. Failure to condition is usually associated with a lack of stimulus *contingency awareness*, defined as the knowledge that the CS predicts the US (Lovibond and Shanks 2002). Contingency awareness is a prerequisite to the development of US expectancy and the emergence of conditioned responses (Grillon 2002; Purkis and Lipp 2001). During conditioning, in the absence of contingency awareness, a signaled shock remains unpredictable. Unfortunately, despite evidence that conditioning is highly dependent on conscious cognitive operations (Dawson and Furedy 1976; Lovibond and Shanks 2002), very few studies provide information on contingency awareness. There is growing evidence, however, that a substantial number of subjects (30%–40%) are unaware of CS-US contingency in aversive conditioning studies (Chan and Lovibond 1996; Haggard 1943; Hamm and Vaitl 1996; Lacey and Smith 1954).

Because conditioning promotes predictability and restricts the range of stimuli that elicit fear, we were interested in investigating how cued fear conditioning deficits would impact conditioned fear responses. We reasoned that individuals unable to verbalize the CS-shock contingency should perceive the shock as unpredictable. Similar to subjects receiving unpaired CS-US, they should exhibit increased context conditioning, compared to individuals aware of stimulus contingency.

This hypothesis was recently tested in a large group of subjects using a differential conditioning procedure in which the CS+, but not the CS−, was reinforced by a shock during conditioning (Grillon 2002). Context conditioning was assessed by testing subjects in the conditioning context either a week or a month following the initial conditioning session. During the first session, subjects' awareness of the CS-shock contingency was assessed after conditioning. *Awareness* was defined as the ability to verbalize the CS-US shock contingency (Dawson and Reardon 1973). Results showed that approximately 40% of the subjects were classified as unaware.[2] Fifty four percent of the subjects were clearly aware. The remaining subjects were not classifiable.

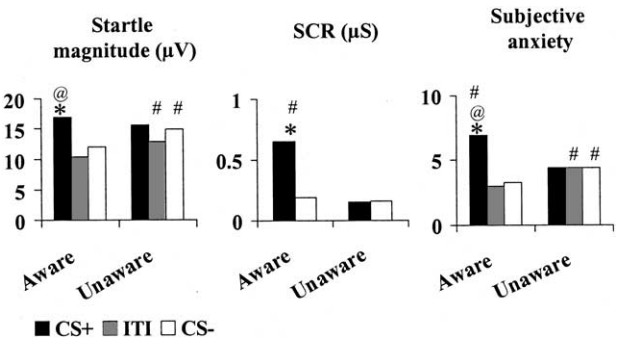

Figure 6. Conditioned responses in the aware and the unaware subjects during the acquisition phase in the first session. Following acquisition, which consisted of the presentation of reinforced CS+ and unreinforced CS−, and a short extinction phase (no delivery of the shock/US), subjects' awareness of the CS-US contingency was assessed. The figure shows results for startle amplitudes during the CS+, CS−, and intertrial interval (ITI) (left panel). The amplitude of the skin conductance response to the CS+ and to the CS- (middle panel) and the subjective rating of anxiety to CS+ and to CS− (left panel) are shown. The subjective rating was a retrospective rating of anxiety of the CS following conditioning. Note that only aware subjects showed differential (i.e., greater) responses to the CS+ and CS−. Significant ($p < .05$) difference in within-subject comparisons between CS+ and CS− are noted * and between CS+ and ITI are noted @. Significant ($p < .05$) between-subject effects are noted #. (Reproduced with permission from Grillon 2002.)

Consistent with the awareness categorization, only the aware subjects acquired differential conditioned responses to the CS+/CS− (Figure 6). They showed greater startle magnitude, skin conductance response, and subjective report of fear ratings to the CS+ compared to the CS−, whereas the unaware subjects had similar responses to both the CS+ and the CS−. These findings reinforce the view that conditioning is a controlled cognitive process, requiring the learning of relationships between the CS and the shock.

The failure to learn that the CS predicted the shock, however, did not prevent the unaware subjects from being anxious. Their fear generalized to all the contextual cues, as suggested by the fact that they showed significantly enhanced physiologic (i.e., startle) and subjective signs of anxiety to CS− and intertrial interval. Thus, while the aware subjects exhibited signs of fear only during the

[2] The relatively high number of unaware subjects could be due to the fact that subjects were not instructed beforehand that they would be asked to identify contingency at the end of the experiment. It is also possible that the startle stimuli interfered with conditioning. Because startle stimuli are delivered during CS+, CS−, and ITI, they may reduce the differentiation between the CS+ and CS−.

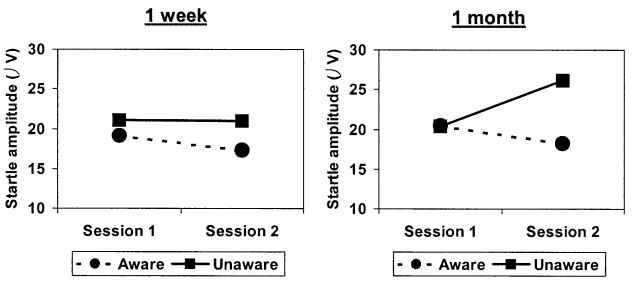

Figure 7. Baseline startle amplitude to acoustic startle stimuli presented before differential aversive conditioning in each of two sessions in aware and unaware subjects returning a week or a month after initial conditioning. Baseline startle did not differ between the two groups during the first session but was greater in the unaware compared to the aware upon returning in the conditioning context, especially in the 1-month group. (Reproduced with permission from Grillon 2002.)

CS+, the unaware subjects were in a more generalized state of anxiety throughout conditioning. In addition, relative to the aware subjects, the startle response of the unaware subjects was potentiated in the second session, compared to the first session (Figure 7). This effect was particularly striking in subjects returning 1 month after initial conditioning. Again, these results are highly similar to that of subjects who were explicitly given unpredictable shocks and showed enhanced signs of anxiety when returning to the conditioning context (Grillon and Davis, 1997).

Moreover, not only did the unaware subjects show more physiologic signs of contextual fear, but they also showed signs of what could be construed as behavioral avoidance of the conditioning context. Unaware subjects were significantly more likely not to return for the second session, compared to the aware subjects. For example, in the 1-month group, only 4 out of 28 aware subjects did not return versus 10 out of 25 unaware subjects who failed to as well. This suggests that individuals who could not predict the shock avoided the conditioning context in higher numbers. This observation, combined with the finding of increased physiologic signs of anxiety (i.e., potentiated startle) in the unaware subjects who did return, is consistent with the two-factor theory of anxiety that views anxiety symptoms as learned responses that are acquired and maintained through a combination of classical/Pavlovian conditioning and avoidance (Mower 1939). An important aspect of these findings, at least as far as human studies are concerned, is the emphasis on context conditioning rather than *explicit cue* conditioning as a model for anxiety.

The results in the unaware subjects are remarkably consistent with animal studies that show that unpredictable aversive stimuli are more anxiogenic (Mineka and Kihlstrom 1978) and lead to more avoidant behaviors (Odling-Smee 1975) than do predictable ones, indicating that this is a broad phenomenon across species; however, a critical aspect of these results is that unpredictability was not predetermined. Shocks were not inherently unpredictable. They were only *perceived* as unpredictable, due to a failure to learn the contingency between the CS and the shock. This suggests a link among associative learning deficits, deviant expectancies for aversive stimuli, enhanced context conditioning, and subsequent behavioral avoidance.

Cued Fear Conditioning and Anxiety

What are the factors that may interfere with contingency awareness or cued fear conditioning? The study by Grillon (2002) showed that the unaware subjects had higher levels of trait anxiety as measured with the Spielberger State and Trait Anxiety Inventory (Spielberger 1983). The differential conditioning study by Chan and Lovibond (1996) also suggests that anxiety interferes with cued fear conditioning. These authors specifically examined the effect of trait anxiety on contingency awareness by dividing subjects into a low trait and a high trait anxiety group before conditioning. Contingency awareness was strongly associated with trait anxiety. Sixty-one percent of the 23 low trait anxious subjects versus only 33% of the 42 high trait anxious subjects were aware of stimulus contingency. Possibly, in vulnerable individuals (e.g., people with high trait anxiety), anxiety or worry caused by shock anticipation may prompt irrelevant thoughts and distract them from monitoring the environment appropriately (Borkovec et al 1991).

Orienting, Conditioning, Autonomic Flexibility, and Risks for Anxiety

Given the role of cognitive process in conditioning, controlled perceptual/attentional behaviors are necessarily engaged during the formation of CS-US associations. The organism needs to orient, pay attention, process information, and rehearse in short-term memory (Dickinson 1980; Rescorla 1980). Deficits at any level of these cognitive operations could interfere with successful cued fear conditioning.

In order for a subject to discover the significance of a CS as a signal for the US, the CS needs to be perceived and processed. Initial processing of stimuli gives rise to an orienting response (OR) that may facilitate the development of CS-US association (Maltzman 1979). Figure 8 shows results that support the role of the OR in contingency awareness. Before the acquisition phase of a differential conditioning procedure, Baas (2001) presented a series of nonreinforced CS+ and CS−. Contingency

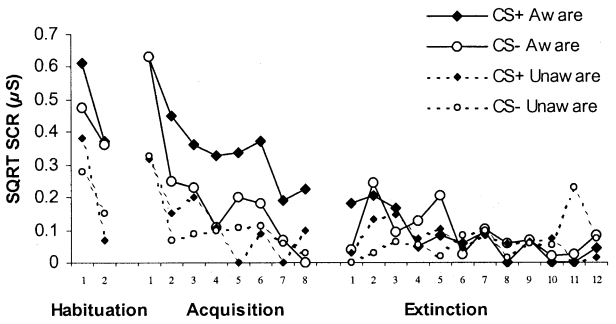

Figure 8. Skin conductance response to the CS during habituation (CS presented without shocks), acquisition, and during extinction in the aware and unaware subjects. Note that the aware subjects showed greater skin conductance responses to the initial presentation of the CS (habituation), suggesting greater orienting to these stimuli, compared to the unaware subjects. Note also that consistent with the data shown in Figure 5, only the aware subjects showed differential conditioning during acquisition. (Reproduced with permission from Baas 2001.)

awareness was assessed at the end of the conditioning procedure. As Figure 8 indicates, the skin conductance response to both CSs before conditioning was greater in the aware compared to the unaware subjects, suggesting greater OR in the former compared to the latter group. Although the skin conductance response remained greater in the aware compared to the unaware subjects throughout the experiment, only the aware subjects showed good CS+/CS− differentiation during acquisition. In fact, the unaware subjects did not show significant differential conditioning. Similar results were obtained in a reanalysis by Grillon (2002). The skin conductance response to the CS presented before the conditioning phase was smaller in unaware subjects, compared to aware subjects. This was not due to the fact that the unaware subjects were autonomically under-responsive. They showed normal SCR to the shocks and to the startling probes.

These results are consistent with results in animals. Selden et al (1991) showed that cortical depletion of noradrenaline impaired cued fear conditioning but enhanced context conditioning. Given the role of the noradrenergic system in selective attention, these findings further stress the role of attentional processes in modulating responses to explicit and contextual cues.

These results emphasize the role of attentional factors in the formation of conditioned responses and the subsequent learning of emotional responses. If the OR is related to the organism's ability to perceive environmental changes, to discriminate among stimuli, and to learn appropriate conditioned responses, deficits in OR could be a risk factor for anxiety or could contribute to the maintenance of anxiety symptoms. The ability to orient to innocuous stimuli appears to be a constitutional characteristic of the individual, emerging early in life. Individual differences in the OR have been related to discrimination learning in children as young as 9 years old (Cousins 1976) and to autonomic conditioning in 3-month-old infants (Ingram and Fitzgerald 1974). Deficits in the OR and in associative learning could have a pervasive impact on behavior, including mental health. A recent trend recognizes the need to integrate models of emotion and cognition on adaptive behaviors. Negative affect and anxiety are hypothesized to bias attentional functions (Mathews et al 1990; Mogg and Bradley 1998). Our studies point to the disruptive role of abnormal attentional operations on emotional regulations, effects that may well start at a very early age.

These statements are consistent with an emerging literature that links attentional regulation and autonomic flexibility to affective processes (Thayer and Lane 2000). Flexible attention to stimulus changes, as reflected by a robust OR, is a prerequisite to adaptive responses to the environment and may be vagally mediated (Porges 1992). Reduced vagal tone has been shown to be associated with impairment in eyeblink conditioning (Tapp et al 1997). It would be informative to examine whether reduced vagal tone leads to cued fear conditioning deficits.

The Hippocampus and Associative Learning Deficits

The hippocampus has been shown to be involved in context conditioning (Kim and Fanselow 1992; Phillips and LeDoux 1992); however, this has been an area of controversy (Fanselow 2000; Gewirtz et al 2000). Several studies indicate that lesions of the hippocampus either do not affect (Gisquet-Verrier et al 1999; McNish et al 1997) or even *increase* context conditioning (Winocur et al 1987). The latter study was based on a previous report by Odling-Smee (1975) that demonstrated that the amount of avoidance to an aversive context was inversely related to the probability that the US followed the CS. In the Odling-Smee (1975) study, rats were given the choice of staying either in a black compartment, where they received shocks, or in an innately aversive white compartment. The black compartment was preferred over the white compartment if the shock was paired with a CS (i.e., was predictable), but it was avoided if the shock was not paired with a CS (i.e., was unpredictable). These results are informative in the light of our results that failure to form CS-US association leads to increased context conditioning and avoidance. Winocur et al (1987) replicated these findings in animals given sham lesions or cortical lesions; however, animals given hippocampal lesions and receiving paired CS-US avoided the black compartment for the white compartment (similar to control animal receiving unpaired CS-US). Apparently, these animals did not learn

that the CS predicted the shock, making the US unpredictable. In this study, lesions of the hippocampus led to an increase, not a decrease, in context conditioning. These results are somewhat consistent with human data in anxious populations. Decreased hippocampal volume and data consistent with a dysregulation of hippocampal function (Bremner et al 1995; Bremner et al 1993; Gurvits et al 1996) are reported in individuals with PTSD. This is accompanied by an increase, not a decrease, in context conditioning (Grillon and Morgan 1999).

Conclusion

The aim of this review was to interpret findings from studies on human anxiety using the startle reflex in light of animal data. Animal studies have identified two neural circuits involving the amygdala and the bed nucleus of the stria terminalis that may be associated with explicitly cued fear and generalized anxiety to threatening contexts, respectively. Threat prompts either a brief period of fear or a more generalized state of anxious apprehension, according to whether the danger is predictable or unpredictable. The link between attentional and emotional operations in the production of conditioned responses has been emphasized. Deficits in orienting may lead to unpredictability and attentional bias toward generalized threat. Potentially, such deficits could contribute to the etiology and maintenance of anxiety disorders. Future studies should examine the relevance of these findings to pathologic anxiety. In particular, one would predict that patients with generalized anxiety disorder would show deficits in cued fear conditioning and a corresponding increase in contextual fear.

It is too early to speculate on the role of associative learning deficits in the etiology and maintenance of anxiety disorders; however, deficits similar to that reported here have been shown in individuals at risk for anxiety disorders. Anxious children and children at risk for anxiety disorders show deficits in declarative memory processes, such as paired-associate learning (Merikangas et al 1999; Pine et al 1999). These deficits could be related to the ability to orient to cues in the environment, which emerges early in life (Cousins 1976; Ingram and Fitzgerald 1974). Clearly, more work needs to be done to address this issue. The present review may provide the impetus to use conditioning procedures to better understand the development of appropriate and inappropriate aversive expectations.

An attempt was also made to identify areas in the literature that need advancement to help clarify our understanding of aversive responses in animals and humans. The defining properties of contextual cues, as opposed to explicit cues, are unclear. The information provided by the cue concerning upcoming aversive events seems critical because it affects predictability. Furthermore, a stimulus/response model based on multiple response systems may be helpful in further distinguishing fear from anxiety. There is a paucity of human psychopharmacological studies of the effects of anxiolytics on fear-potentiated startle. Such studies are greatly needed to better understand the neuropharmacology of cued and contextual fear; however, issues concerning the measurement of fear-potentiated startle (ratio vs. difference scores) when baseline responses are affected by the treatment should first be addressed. In addition, the anxiolytic effects of drugs on fear-potentiated startle should be distinguished from the side effects of drugs (e.g., sedation, reduced attention).

Finally, human studies on anxiety should further rely on conditioning experiments. Conditioning studies provide a clear-cut link with the animal literature. They also provide a framework to study the development of aversive expectation and the interplay between cognitive and emotional processes during learned fear; however, as the results presented in this review point out, an understanding of conditioned responses in humans requires the analysis of both cued fear and context conditioning.

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